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Three-dimensional structure of the tiny bacterium *Pelagibacter ubique* studied by cryo-electron tomography

D. Nicastro,* C. Schwartz,* J. Pierson,* J-C. Cho**, S.J. Giovannoni**, and J. R. McIntosh*

* Boulder Laboratory for Three-Dimensional Electron Microscopy of Cells, University of Colorado, Boulder, Colorado 80309-0347
**Department of Microbiology, Oregon State University, Corvallis, OR

The marine α-proteobacteria clade *Pelagibacter* (SAR11) has intrigued scientists since their discovery in the late 1990's [1], because they are among the smallest autonomously replicating cells known, yet they are one of the most ubiquitous and numerically abundant microbial groups on the planet [2]. Cryo-electron tomography has allowed us to characterize the cellular organization of these tiny cells, providing new insights into their biology. The cells were rapidly frozen by plunging into liquid ethane and store in liquid nitrogen. 38 single-axis tilt series of vitrified cells have been recorded on a Tecnai F-30 microscope, using the microscope control program, SerialEM. Individual tilt series images have then been aligned, reconstructed, analyzed and visualized by modeling or volume-rendering, using the IMOD [3], EM [4] and AMIRA software packages (TGS, Mercury Computer Systems, San Diego, CA). The three-dimensional analysis has shown that the total cell volumes of *Pelagibacter ubique* strain HTCC1062 range from 0.025 – 0.045 μm^3 when they are actively growing on a natural seawater medium [5]. The periplasmic space occupies 25% of the cell volume in log-phase cells and up to 35% in stationary phase. The nucleoid takes up about half of the intracellular volume, and in most cells it was not centrically positioned. It lies close to the inner membrane on the convex side of the cell. The remaining cytoplasm seems to have a relatively high ribosomal content, a surprising observation for a small bacterium with division times greater than one day [5]. We have also found that a few cells formed numerous long filaments, possibly type-IV pili, during the time of their cell division. These findings indicate that novel insights about the organization and macromolecular structure of small, yet ecologically important organisms will arise from studies of this kind.

References

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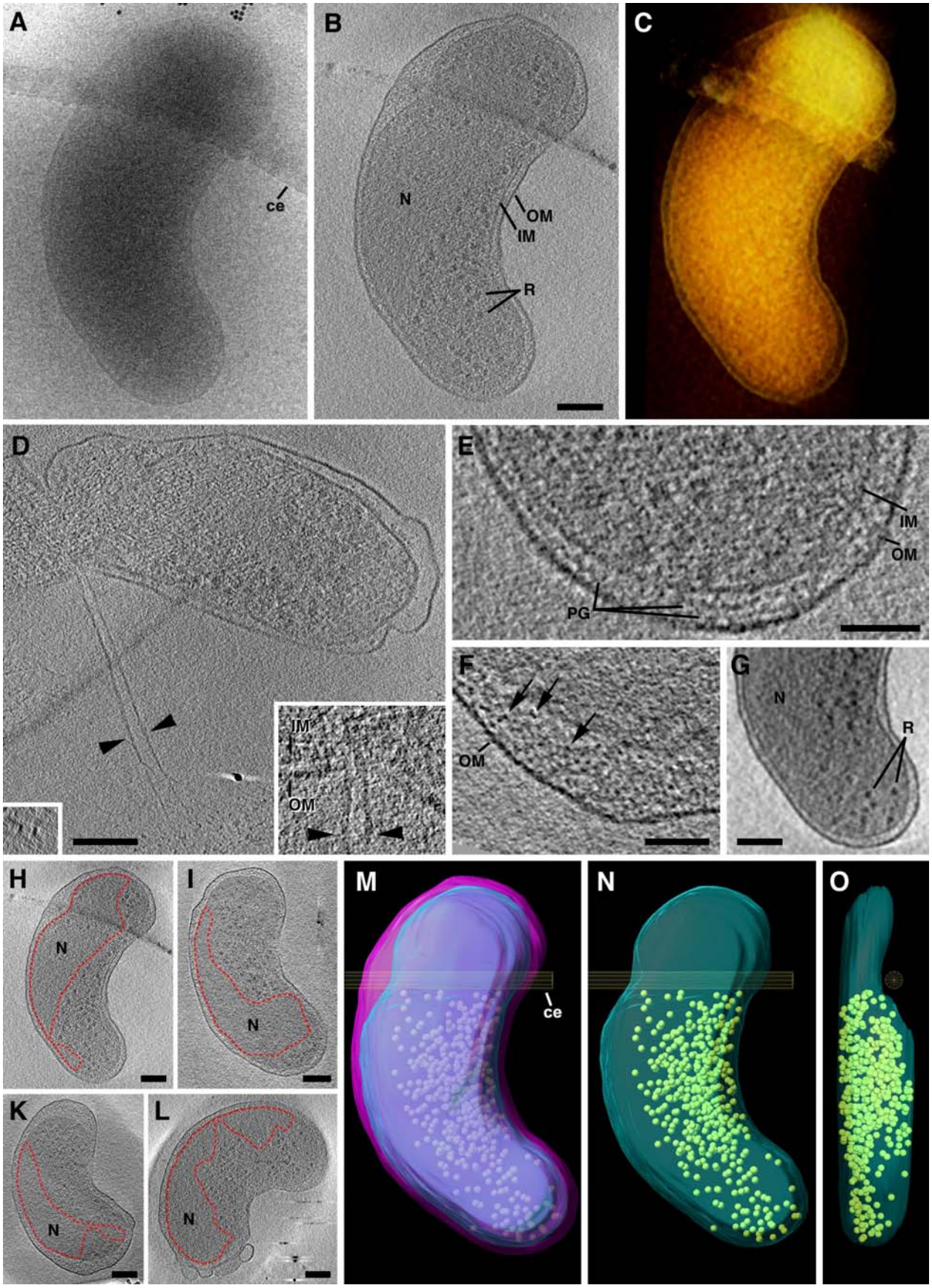
Fig. Electron tomography of intact frozen-hydrated cells of *Pelagibacter ubique*. A) Original micrograph (0°-projection) from a tilt series; at the top the bacterium sits on the carbon support film (*ce*). B) Central xy-slice of the tomographic reconstruction corresponding to (A) showing the subcellular organization. C) Volume-rendered representation of the bacterium in (B). D) Tomographic slice of another cell with two pilus-like filaments (*arrowheads*); left inset) both filaments in cross-section; right inset) basal region of the filaments, showing that they pass through the outer membrane (*OM*). E, F) Periplasmic space with the peptidoglycan-layers (*PG*) (E) and electron dense particles (*arrows*) (F). G) Lower half of the same cell as in (B) after denoising to increase the contrast of the ribosome-like particles (*R*). H-L) Central xy-slices of four different cells; note the outlined nucleoids (*N*) on the convex side of the cells. M-O) Surface-rendered model of the bacterium in (B); (O) is the side view of (N); the outer membrane is contoured in *pink*, the inner membrane in *blue* and the ribosome-like particles in *green*. (*ce*: carbon edge, *IM*: inner membrane, *N*: nucleoid, *OM*: outer membrane, *PG*: peptidoglycan-layer, *R*: ribosome-like particle). Bars: (A-D,G-L) 100 nm; (E,F) 50 nm.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